Dissemination of a recombinant multidrug-resistant *Streptococcus pneumoniae* clone Sweden^{15A}-ST63 with serotype 8 in Spain

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1. Background

Multidrug resistant (MDR) serotype 8 pneumococci showing resistance to erythromycin, clindamycin, tetracyclines, and ciprofloxacin, have been detected since 2004 in Spain as cause of invasive disease in adult patients.

2. Methods

Among 932 invasive serotype 8 pneumococci received at the Spanish Reference Laboratory in the 1997-2010 period, 113 (12.1%) were multidrug resistant. These MDR-serotype 8 isolates were typed by PFGE/MLST and PCR-RFLP analysis of PBPs 1A, 2B and 2X. Resistance genes (*ermB, mefA/E and tetM*) were detected by PCR. Changes in ParC, ParE and GyrA were studied by PCR-RFLP analysis. Nucleotide sequencing of *pbps* and *parC, parE* and *gyrA* were performed.

3. Results

The first MDR-serotype 8 was detected in 2004 in Madrid, and then spread to other geographical areas. All MDR-serotype 8 were isolated from adults and most of them were men (78.8%). MDR-serotype 8 isolates accounted for 20.2% of invasive serotype 8 in 2004-2010 period. All MDR-serotype 8 isolates were genetically related to the Sweden^{15A}-ST63 PMEN clone by PFGE and MLST (ST63), had identical PCR-RFLP profiles of PBP, harbored *ermB* and *tetM* genes, and had a ParC-S79F change. Forty-six isolates having ciprofloxacin MIC —16 mg/L had additional GyrA changes (S81F or E85K). The sequence of *pbp2x* gene of the MDR-serotype 8 clone was identical to those of the antibiotic susceptible ST53-serotype 8 isolates. Whereas pbp2b gene sequence of MDR-serotype 8 isolates was identical to those of Sweden^{15A}-ST63 PMEN-clone. One of the recombinational points was found into the *pbp1a* gene.

4. Conclusions

A fluorquinolone- and multidrug-resistant serotype8 clone (ST63) emerged in 2004 in Spain and spread over the country causing invasive disease in adults. A genetic interchange of both, *pbp2x* and capsular genes from a donor ST53-isolate to a ST63 pneumococci could originate the recombinant serotype 8-ST63 clone.

Keywords: Epidemiology, MLST, multidrug-resistance.

Heteroresistance to fosfomycin is predominant in *Streptococcus pneumoniae*

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ABSTRACT:

Fosfomycin targets the first step of peptidoglycan biosynthesis in *Streptococcus pneumoniae* catalyzed by UDP-N-acetylglucosamine enolpyruvyltransferase (MurA1). We investigated whether heteroresistance to fosfomycin occurs in *S. pneumoniae*. We found that of 11 strains tested all but one (Hungary^{19A}) displayed heteroresistance.

Hungary^{19A} differs from the other strains by a single amino acid substitution in MurA1 ($Ala_{364}Thr$). To test whether this substitution confers structural changes leading to catalytic pocket modification high-resolution crystal structures of MurA1 in one representative heteroresistant strain (D39) and in the non-heteroresistant strain Hungary^{19A} have been solved. We found no relevant structural differences between MurA1 of the two strains. Furthermore, the heteroresistant phenotype of strain D39 was not changed upon introduction of the amino acid substitution $Ala_{364}Thr$ by site-directed mutagenesis indicating that, besides MurA1, another factor within the genome is involved in the heteroresistance phenotype.

Our results reveal that heteroresistance to fosfomycin is the predominant phenotype in *S. pneumoniae* due to the presence of MurA1 thus providing a caveat for any future use of fosfomycin in the treatment of pneumococcal infections.

Keywords: fosfomycin, heteroresistance, MurA.